MECHANISMS REGULATING APPETITE AND ENERGY BALANCE IN POULTRY [1,2]

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ABSTRACT

Intensive selection by poultry breeders for economically important production traits such as growth rate and meat production has led to significant changes in correlated responses such as feed intake and energy metabolism. For example, the modern commercial broiler, selected for rapid growth and enhanced breast meat yield, does not adequately regulate voluntary feed intake to achieve energy balance and a constant body weight. When provided unrestricted access to feed, broilers will tend to over eat leading to an excessive accumulation of energy (fat) stores and a variety of health-related problems. A series of highly integrated regulatory mechanisms exists for control of appetite and energy balance involving complex interactions between peripheral tissues and the central nervous system. Within the central nervous system, the brainstem and the hypothalamus play critical roles in the regulation of feed intake and energy balance. Genes encoding key regulatory factors such as hormones, neuropeptides, receptors, enzymes, transport proteins, and transcription factors constitute the molecular basis for regulatory systems that derive from integrated sensing, signaling, and metabolic pathways. We do not yet have a complete understanding of the genetic basis for this regulation in poultry. A better understanding of the genes associated with controlling feed intake and energy balance and how their expression is regulated by nutritional and hormonal stimuli will offer new insights into current poultry breeding and management practices.

Keywords: Poultry, appetite, satiety, feed intake, energy balance, body weight, leptin, melanocortin system, genetic regulation.

INTRODUCTION

Poultry breeders have intensively selected meat-type birds over many generations with specific emphasis on increasing growth rate (body weight) and meat production. Increased body size in commercial chicken and turkey lines has been accompanied by unintended changes in correlated traits such as voluntary feed intake and energy storage. For example, modern commercial broiler strains selected for rapid growth and high meat yields have lost the ability to regulate voluntary feed intake commensurate with energy requirements. As a result, these birds must be fed a limited amount of feed to avoid over consumption that can lead to excessive accumulation of energy stores (fat tissue), an undesirable body weight/composition, and a series of health-related complications (i.e., leg problems, reduced reproductive efficiency, etc.).

The amount of feed consumed is a fundamental factor that determines the rate of growth and body composition in poultry. In animals, body weight is carefully controlled throughout development by adjustments to feed intake and energy expenditure (McMinn et al., 2000). There have been a number of excellent reviews regarding the regulation of feed intake by the central nervous system and peripheral tissue mechanisms in poultry (Sykes, 1983; Denbow, 1994; Kuenzel, 1994; Kuenzel et al., 1999; Richards, 2003). A good example of an adaptive mechanism regulating feed intake involves laying hens fed different levels of dietary energy (Sykes, 1983). These birds make adjustments in their voluntary feed intake to maintain a constant energy intake. This adaptive response indicates an ability to ‘sense’ dietary energy concentration and match that with energy requirements. During the past decade, it has become increasingly clear that the regulation of feed intake and the regulation of energy balance in poultry

are linked by a highly complex and integrated series of neural and endocrine networks (Richards, 2003). This linkage involves mechanisms that sense the presence/absence of feed in the gut and the level of body energy stores (fat) coupled with signaling pathways in the central nervous system that bring about appropriate changes in feed intake and energy expenditure in order to maintain body weight. Modern poultry production systems strictly limit feed provided to birds throughout their lifecycle. This practice may not be the most efficient method to achieve optimal body weight and composition in commercial flocks because it does not control all of the associated changes in energy expenditure that are linked to appetite. As a result energy metabolism can become imbalanced leading to excessive energy (fat) storage despite the imposed feed restriction.

Feeding behavior and energy balance are basic processes crucial to the survival of all animals. Therefore, it is logical to assume that the regulatory mechanisms governing these processes in birds and mammals would involve highly conserved neural and endocrine sensing and signaling networks as well as similar neuroanatomical sites (Kuenzel, 1994; Kuenzel et al., 1999). In fact, much of what has been discovered recently concerning the regulation of appetite and energy balance has come from studies involving mammalian species. Despite many apparent similarities, it would be incorrect to assume that these findings are directly applicable to poultry. As avian-based studies continue, new insights are showing that the regulation of appetite and energy balance in birds may differ in specific key areas from that observed in mammals (Richards, 2003). Some of the similarities and differences in regulatory mechanisms are discussed in this review.

ADIPOSE TISSUE: AN ENDOCRINE ORGAN

An important concept developed during the past decade is the classification of adipose tissue as an organ that is capable of synthesizing and secreting a variety of endocrine factors (Milner, 2004). Traditionally, adipose tissue has been regarded primarily as a depot for the storage of excess energy in the form of triglycerides. **Table 1** lists a number of factors synthesized and secreted by adipose tissue. These factors fall into two general categories: 1) factors previously recognized as non-adipose signals that have general functions in various organ systems throughout the body (i.e., cardiovascular, immune, reproductive, etc.) and 2) those that have adipose-specific endocrine functions. To date there has been very little investigation of avian adipose tissue from this perspective.

The discoveries of leptin and its receptor and their subsequent characterization as the molecular basis for the regulatory system linking the sensing of peripheral energy stores with control of feed intake via the central nervous system clearly emphasized an important endocrine role for adipose tissue in mammals (Friedman and Halaas, 1998). Leptin was originally identified as the product of the mouse *ob* gene that is expressed predominantly in adipose tissue, but also by a variety of other tissues to a lesser extent (Zhang et al., 1994). It was found to play a role in the regulation of appetite, energy expenditure and maintenance of body weight through its actions on specific hypothalamic sites as part of a negative feedback control system (Friedman and Halaas, 1998). The signaling function of leptin was subsequently found to require the expression of specific leptin receptors (Tartaglia et al., 1995). Leptin and its cognate receptor, together, constitute the leptin system.

A considerable amount of experimental data has been collected concerning the expression, actions, and functional importance of leptin in a number of mammalian species (Friedman and Halaas, 1998; Friedman, 2002). Although there is evidence that strongly suggests a conserved role for leptin and its receptor in regulating body weight and energy balance across a number of different mammalian species, considerably less is known about avian leptin and its functions. To date, there have been two reports of the cloning and sequencing of a chicken leptin gene (Taouis et al., 1998; Ashwell et al., 1999). The predicted amino acid sequence shows high homology with mammalian (mouse) leptin proteins. Peripheral (ip) and central (icv) administration of recombinant leptin protein to birds reduced food intake in some trials (Dridi et al., 2000; Denbow, et al., 2000; Taouis et al., 2001; Lohmus et al., 2003) or was without effect in others (Bungo et al., 1999). Leptin protein levels in plasma and tissue (liver and fat) samples from chickens have been analyzed using specific immunoassay techniques (Richards et al., 1999; Taouis et al., 2001). Despite these findings, considerable doubt has been cast on the validity of the gene sequence reported for chicken leptin and on the analysis of leptin gene expression (Friedman-Einat et al., 1999). In fact, Friedman-Einat et al. (1999) and others using a variety of molecular techniques failed to find any evidence for the reported chicken leptin gene sequence in mRNA reverse transcribed from liver or fat tissue collected from several chicken strains, turkey, goose or Japanese quail or in chicken genomic DNA samples. Furthermore, an initial report of mapping the chicken leptin gene to a microchromosome (Pitel et al., 1999) was later determined to be incorrect (Pitel et al., 2000). A search of the draft sequence of the chicken genome also fails to indicate the existence of sequence corresponding to that reported for chicken

leptin gene. Therefore, in order to unequivocally establish the role of leptin as a signal of energy stores in birds, it is imperative that the entire leptin gene sequence and consistent leptin gene expression measurements in different tissues (especially liver and adipose tissue) be completed and fully verified for different poultry species. Only then will it be possible to ascribe a definitive role to the putative avian leptin hormone as a signal of peripheral energy stores to the central nervous system for the long-term regulation of feed intake and energy balance in poultry.

In contrast, the leptin receptor gene has been clearly identified and characterized for both chickens and turkeys (Ohkubo et al., 2000; Horev et al., 2000; Richards and Poch, 2003). Based on the deduced amino acid sequence, it appears that the avian leptin receptor is quite similar to the mammalian receptor. To date only the 'long form' receptor has been fully characterized in birds, although there is some preliminary indication for the existence of similar short or truncated forms of the receptor as have been characterized in mammalian species (Tartaglia et al., 1995). The long form is capable of full signaling in response to bound leptin. Moreover, sequence analysis of the putative leptin-binding domain indicates that the avian leptin receptor is capable of binding mammalian leptin proteins (Richards and Poch, 2003). Similarly, the leptin-binding domain of the human leptin receptor has recently been shown to bind non-human leptin proteins, including recombinant chicken leptin (Sandowski et al., 2002). This may help explain the reported effectiveness of mammalian recombinant leptin proteins (viz., human and sheep) in reducing food intake when administered to chickens (Denbow et al., 2000; Taouis et al., 2001). Based on these findings, it is clear that birds express a functional leptin receptor in both central nervous system and peripheral tissue

sites (Ohkubo et al., 2000; Horev et al., 2000; Richards and Poch, 2003). Recombinant chicken leptin was reported to attenuate ovarian follicular apotosis in hens during a five day fast (Paczoska-Eliasiewicz et al., 2003). This effect was mediated by the expression of leptin receptors in the ovary as was reported previously (Ohkubo et al., 2000). Together these findings suggest the potential for a functional leptin signaling system in poultry.

PEPTIDE SIGNALING MOLECULES

Table 2 lists a number of peptides that have been identified and studied in poultry and mammalian species with respect to their effects on appetite and energy balance. These signaling molecules function in peripheral and/or central sites to activate specific neural circuits that effect changes in feed intake as well as in energy metabolism. The signaling molecules listed in **Table 2** have been studied in both mammals and birds, either by injection into central nervous system or peripheral sites (Woods et al., 1998; Denbow, 1994; Kuenzel, 1994). In avian studies mammalian analogues have been tested and some avian homologues have also been studied. Specific examples of some well-studied avian signaling molecules include: neuropeptide Y (NPY), proopiomelanocortin (POMC) and its product alpha-melanocyte stimulating hormone (α-MSH), cholecystokinin (CCK), and bombesin (Denbow, 1994; Kuenzel, 1994; Jensen, 2001). It is clear from the data summarized in **Table 2** that despite the conserved nature of the peptide signaling molecules between birds and mammals, there are differences in the function of specific peptides. For example, peptide YY (PYY) and pancreatic polypeptide (PP) suppress appetite in mammals, whereas they are both potent orexigenic agents in birds (Kuenzel et al., 1987; Ando et al., 2001). On the other

hand, melanin concentrating hormone (MCH), orexins (A & B), galanin, and motilin all are potent orexigenic agents in mammals, but are without effect in chickens (Furuse et al., 1999; Ando et al., 2000; Ohkubo et al., 2002). Thus, the fact that birds and mammals have common signaling peptide molecules does not mean that they also share a common function. It will be necessary to extensively study the expression of genes that encode these substances under various physiological conditions to better understand the specific function(s) of these signaling agents in birds.

For each of these signaling molecules to be active, specific receptors that recognize and bind them must be produced at sites of action. In the case of poultry, a number of these specific receptors have been identified and characterized either at the gene and/or protein level in both peripheral and central sites. Some examples include: leptin receptor (Ohkubo et al., 2000; Horev et al., 2000; Richards and Poch, 2003), NPY receptors (Holmberg et al., 2002), melanocortin receptors (Takeuchi and Takahashi, 1998), the ghrelin receptor also known as the growth hormone secretagogue receptor (Tanaka et al., 2003), and others. In general, the avian homologues for both signal and receptor molecules appear to be somewhat similar in structure to those characterized in mammalian species. However, a definitive assessment of similarities and differences, especially with respect to bioactivity, awaits more information to be obtained from on-going efforts to identify and fully characterize the avian gene homologues that encode these important molecular species. The site of production of a particular peptide signal or its cognate receptor may determine its specific function in regulating appetite and energy balance.

SHORT-TERM REGULATION OF APPETITE

In animals, the drive to feed ensures that immediate energy and nutritional requirements are met from meal-to-meal, as food is available for consumption. Appetite control is crucial to ensuring optimal nutrition and achieving full potential for growth and development in poultry. Control of feed intake in the short-term (i.e., meal-to-meal) involves hormonal and neural signals that originate primarily in the gut, but also in other sites such as the pancreas and liver. The presence of food or specific nutrients in the gastrointestinal tract stimulates the release of a number of different peptides that control gut motility and secretion, as well as serving as satiety signals to the brain. Short-term regulation of feed intake thus involves a satiety response just prior to or during feed consumption with satiety signals (peptides) originating in the gut transmitted to the brainstem via the activation of neural (vagal) afferent pathways or via secretion of signaling substances directly into the bloodstream. Two types of signals produced by the gastrointestinal tract have been proposed: those that stimulate feeding behavior such as ghrelin and those that inhibit it such as CCK and bombesin (Woods et al., 1998; McMinn et al., 2000; Jensen, 2001; Blevins, et al., 2002). Examples of both types of satiety signals have been reported in poultry species.

The recently discovered peptide hormone, ghrelin, has been reported to stimulate feeding in mammals (Wren et al., 2000). Ghrelin is produced by chicken proventriculus and it may modulate feeding behavior in addition to functioning as a potent pituitary releasing factor for growth hormone (GH) through the GH secretagogue receptor (Furuse et al., 2001; Ahmed and Harvey, 2002; Kaiya et al., 2002; Wada et al., 2003; Baudet and Harvey, 2003). Interestingly, ghrelin was shown to inhibit feed intake when administered centrally (icv) to chickens (Furuse et al., 2001; Saito et al., 2002).

This seemingly contradictory effect may actually reflect its potent GH releasing capability, since GH has been shown to inhibit feeding in chickens (Rosebrough et al., 1991). However, it may also indicate a genuine species difference in the function of this gut-derived peptide. Ghrelin genes from chickens and turkeys and other avian species have now been sequenced and characterized. The presence of unique sequence variations have been observed in the region of the gene corresponding to the 5' untranslated region of the mRNA (**Figure 1**). Leghorn chickens contain an 8 bp insert that is not present in broilers and this difference may be a useful genetic marker that distinguishes egg-type from meat-type birds, two groups with markedly different appetites. Turkeys also exhibit additional inserted sequence at the junction of the first two exons (**Figure 1**). The significance of these sequence variations with respect to gene function is not known. However, the predicted and actual amino acid sequence of the ghrelin in birds shows significant conservation (**Figure 2**) especially in the amino-terminal region of the mature peptide hormone that contains an important site (serine 3) that is modified by esterification of a fatty acid (Kaiya et al., 2002). The discrepancy in function between avian and mammalian ghrelin peptides with respect to feed intake regulation is not readily apparent from its structure (gene or protein) and thus remains to be elucidated. Moreover, any additional functions for avian ghrelin other than its demonstrated GH-releasing activity are not currently known. Expression and tissue localization of the ghrelin receptor (GH secretagogue receptor) must also be taken into account when attempting to understand the function(s) of ghrelin in birds.

Cholecystokinin (CCK), a potent inhibitor of feeding, has been well studied in birds as has bombesin and its related peptides (Denbow, 1994; Kuenzel, 1994; Jensen,

2001). Not only does CCK stimulate gastric emptying and the release of pancreatic enzymes to aid in the digestion of feed, but it also functions as a satiety signal to the brainstem capable of depressing appetite and hence the drive to feed. Generally, these signals act locally to effect changes in gut secretions and motility as well as acting on afferent fibers of the vagus nerve that innervate the gut, the liver and the pancreas and connect with the brainstem satiety center. Their effects are relatively short-lived and components of the signaling system are found both in the gut and the brain. In addition, CCK, ghrelin, bombesin, and other peptide satiety signals are also released into the bloodstream where they can find their way into the central nervous system to activate specific neural pathways that affect appetite. Since these signals are only active for short periods of time, they are effective in regulating meal size but may not be capable of producing long-term changes in energy balance or body weight.

METABOLIC PATHWAYS AND METABOLITE SIGNALING

Peripheral tissues carry out the functions of flux, storage, mobilization and utilization of fuels under hormonal control with sympathetic and parasympathetic nervous system inputs (Berthoud, 2002). Circulating levels of metabolites (triglycerides, glucose, free fatty acids, amino acids) might also serve as signals of energy/nutritional status to the brain. In this way, metabolic pathways and metabolites produced by them would be integrated into the regulatory scheme for feed intake and energy metabolism.

When energy from feed is consumed in excess of the quantity needed to meet requirements (a state of positive energy balance), it is generally stored in the form of triglycerides which are products of the lipogenic metabolic pathway (**Figure 3**). In birds, the major site of lipogenesis (i.e., the *de novo* synthesis of triglycerides from glucose) is

253 the liver (Hillgartner et al., 1995), with adipose tissue serving primarily as a repository
254 for storing accumulated triglycerides (**Figure 3**). The genes encoding the enzymes
255 involved in fatty acid synthesis are subject to regulation by the major metabolic
256 hormones, insulin, glucagon and thyroid hormone (T_3). Insulin and T_3 induce, whereas
257 glucagon inhibits, the expression of lipogenic genes (Hillgartner et al., 1995). Thus, in
258 positive energy balance when the levels of circulating insulin and T_3 would be
259 increased, lipogenic gene expression in liver is increased leading to an enhanced
260 production of triglycerides and increased adipose tissue storage. Also, expression of a
261 gene encoding the key transcription factor, sterol regulatory element binding protein 1c
262 (SREBP-1), is regulated positively by insulin and T_3 and negatively by glucagon
263 (Gondret et al., 2001). This single transcription factor, which is highly expressed in the
264 liver of birds, regulates the expression of the major lipogenic enzyme genes producing a
265 coordinated response in gene expression for this metabolic pathway (Gondret et al.,
266 2001). In this way an entire metabolic pathway (e.g., lipogenesis) responds to changes
267 in energy balance and, in doing so, directly determines changes in the size of adipose
268 tissue mass that is comprised largely of stored triglycerides, the products of lipogenesis.
269 Changes in adipose tissue mass due to triglyceride accumulation would presumably
270 influence leptin (and presumably other adipocyte endocrine factors) gene expression
271 that would, in turn, affect feed intake to bring about appropriate adjustments in energy
272 balance. Conversely, restricting the amount of feed given to broiler breeder pullets
273 significantly affected lipogenic gene expression and metabolic hormone profiles as the
274 birds transitioned into egg production (Richards et al., 2003). It has recently been
275 reported that elevated circulating triglyceride levels in rodents can cause leptin

resistance mediated by impaired transport (across the blood-brain barrier) of leptin to the hypothalamus (Banks et al., 2004), while stimulating hypothalamic neurons that produce specific feeding-stimulatory peptides thus promoting an orexigenic response (Chang et al., 2004). Leptin gene expression in birds has been reported in both liver and adipose tissue, with liver being the predominant site of expression (Taouis et al., 1998; Ashwell et al., 1999). This has been suggested to reflect the prominent role of the liver in lipogenic activity in birds. Thus, a link would be predicted between metabolic and endocrine pathways involved in feed intake and energy balance.

AMP-ACTIVATED PROTEIN KINASE AND CELLULAR ENERGY STATUS

Recent findings in mammals have indicated leptin and other anorexigenic substances reduce appetite by inhibiting an enzyme that 'senses' cellular energy levels in hypothalamic neurons (Minokoshi et al., 2004; Unger, 2004). This evolutionarily conserved enzyme, AMP-activated protein kinase (AMP-kinase) is activated (phosphorylated) under conditions of low cellular energy as determined by the AMP/ATP ratio (**Figure 3**). In this context, AMP-kinase serves a 'fuel gauge' that responds to fluctuations in cellular energy levels (AMP/ATP ratio), as well as to specific nutrients such as glucose and fatty acids and hormones such as leptin. When active, AMP-kinase acts on (phosphorylates) acetyl-CoA carboxylase, the rate-limiting enzyme involved in the production of malonyl-CoA used for fatty acid biosynthesis, and causes a reduction in this reaction (**Figure 3**). The conversion of acetyl-CoA to malonyl-CoA catalyzed by acetyl-CoA carboxylase is a crucial step in energy metabolism because it links fatty acid metabolism with carbohydrate metabolism through a common intermediate metabolite, acetyl-CoA. In general, AMP-Kinase activates catabolic (ATP-

generating) pathways such as fatty acid oxidation while inhibiting energy consuming pathways such as fatty acid synthesis. Leptin, by inhibiting AMP-kinase activity in the hypothalamus, causes the activity of acetyl-CoA carboxylase to be increased leading an increased level of malynoyl-CoA which promotes anorexigenic signaling and reduced feed intake (Minokoshi et al., 2004; Unger, 2004). The accumulation of malanoyl-CoA, caused by either increasing the activity of acetyl-CoA carboxylase or by inhibiting the activities of downstream enzymes such as fatty acid synthase or stearoyl-CoA desaturase-1 (**Figure 3**), inhibits the activity of carnitine palmitoyl transferase-1 (CPT-1), a transport protein located in the inner mitochondrial membrane that catalyzes the rate-limiting step in fatty acid oxidation (Hu et al., 2003 Dobrzyn et al., 2004). Thus, hypothalamic AMP-kinase activity and the level of malanoyl-CoA serve as indicators of cellular energy status which mediate changes in appetite and fuel utilization. AMP-kinase also functions in peripheral tissues such as liver and skeletal muscle to bring about changes in energy metabolism. To date there has been no investigation of the roles of AMP-kinase and malonyl-CoA in regulating feed intake in birds, although avian gene sequence databases indicate the existence of AMP-kinase subunit genes.

UNCOUPLING PROTEIN AND ENERGY EXPENDITURE

Heat production or thermogenesis is an important component of energy expenditure used by animals to maintain core body temperature in cold environments. Non-shivering or adaptive thermogenesis is one method of generating heat. It involves the uncoupling of oxidative phosphorylation in mitochondria with energy being produced as heat instead of being converted to its chemical form, ATP. This is achieved by the actions of at least one member of a specific family of mitochondrial membrane

transporter proteins called uncoupling proteins (UCPs) that dissipate the proton gradient across the inner mitochondrial membrane (Himms-Hagen and Harper, 2001). UCPs promote the transport of protons into the mitochondrial matrix resulting in a reduction in the electrochemical potential established by a proton gradient (Garvey, 2003). The ensuing increase in oxidation of substrates leads to the production of energy in the form of heat with fatty acids being the predominant substrate utilized for thermogenesis (Himms-Hagen and Harper, 2001). To date five UCPs have been identified and characterized in mammals (Himms-Hagen and Harper, 2001; Garvey, 2003), whereas, a single UCP has been identified in birds (Raimbault et al., 2001; Vianna et al., 2001; Evock-Clover et al., 2002). It appears that the avian UCP, which is expressed predominantly in skeletal muscle, most closely resembles mammalian UCP3 (Evock-Clover et al., 2002). Furthermore, it has been proposed that rather than an uncoupling function, UCP3 may in fact be involved with shuttling fatty acid anions outward across the inner mitochondrial membrane, and thus it might play a role in promoting fatty acid oxidation by reducing the accumulation of acyl-CoAs within the mitochondria (Himms-Hagen and Harper, 2001; Evock-Clover et al., 2002; Garvey, 2003). Since ATP is produced by oxidation of fatty acids, the net result of an increased level and activity of UCP3 would be to increase energy production consistent with a role for UCP in the regulation of lipid utilization as a fuel substrate (Collin et al. 2003a). Although, there have been reports in mammals that UCP gene expression is increased by leptin, no effect of leptin on skeletal muscle UCP gene expression, either in negative energy (fasted) or positive energy (Fed) balance states was found in chickens (Evock-Clover et al., 2002). However, glucagon, T_3, and cold-exposure were shown to up-regulate avian

UCP gene expression in skeletal muscle (Raimbault et al., 2001; Toyomizu, et. Al., 2002; Collin et al., 2003b; Collin et al., 2003c), whereas UCP gene expression level was negatively correlated with circulating insulin levels in fed and fasted chickens (Evock-Clover et al., 2002). Moreover, it has been suggested that UCP expression in skeletal muscle in both mammals and birds responds positively to the level of circulating free fatty acids (Himms-Hagen and Harper, 2001; Evock-Clover et al., 2002). Clearly, more study is required to determine what role UCP might play in energy metabolism in poultry.

LONG-TERM REGULATION OF ENERGY BALANCE

In mammals, experimental findings suggest that body energy stored in the form of adipose tissue is tightly regulated (McMinn et al., 2000). Adaptive changes in both feed intake and energy expenditure contribute to homeostasis of body energy stores and the maintenance of a constant body weight. In addition to meeting immediate energy demands, feed intake can be adjusted to ensure that energy and nutrients are stored in anticipation of periods of high demand or periods of feed shortage. The hypothalamus contains multiple peptidergic neuronal pathways that are involved in the regulation of feed intake and energy homeostasis. These pathways can be divided into two basic categories, anabolic and catabolic (Woods et al., 1998). Stimulation of one set of neurons (NPY-expressing) mediates a net increase in energy intake and storage (anabolic pathways), whereas stimulation of the other set (POMC-expressing) results in a net decrease in energy intake and storage (catabolic pathways). In mammals, changes in the circulating level of leptin and possibly insulin signal the hypothalamus to

effect long-term changes in energy balance by activating and/or inhibiting specific anabolic and catabolic pathways (**Figure 4**).

Chickens, like mammals, express similar genes encoding neuropeptides such as NPY and POMC (α-MSH is derived from proteolytic processing of the POMC precursor protein) that form anabolic and catabolic peptidergic effector circuits in the hypothalamus. The NPY gene has been cloned and sequenced in chickens and its localized expression in the brain determined (Blomqvist et al., 1992; Wang et al., 2001). NPY gene expression in the brain responds to changes in energy status caused by fasting and feed restriction of chickens (Boswell et al., 1999). Moreover, NPY has been shown to be a potent orexigenic agent in chickens when administered centrally (Kuenzel et al., 1987; Kuenzel and McMurtry, 1988). Specific NPY receptors (Y1 and Y5) have been reported to mediate NPY effects on feeding behavior in chickens (Holmberg et al., 2002). The POMC gene has been identified and sequenced in chickens and it was shown to produce bioactive α-MSH that appears to play an important role in regulating feed intake in chickens (Takeuchi et al., 1999; Gerets et al., 2000; Kawakami et al., 2000). Central (icv) administration of α-MSH strongly inhibits feed intake in chickens (Kawakami et al., 2000). Not only are melanocortin receptors expressed in central sites, but they are also widely expressed in peripheral tissues of chickens as well (Takeuchi and Takahashi, 1998). The agouti-related peptide (AgRP) gene homologue has been identified, cloned, and sequenced in chickens and expression of this naturally occurring antagonist of melanocortin action was reported to be widespread in central and peripheral tissues in chickens (Takeuchi et al., 2000). AgRP serves an antagonist of α-MSH in chickens, as it does in mammals, by binding to

specific melanocortin receptor subtypes (MC3-R and MC4-R). AgRP is orexigenic in layer-type chickens, but not broilers, when administered intracerebroventricularly (Tachibana et al., 2001). Based on these observations, it was concluded that the MC4-R might function in the regulation of feed intake and energy balance in chickens as this receptor subtype has been postulated to do in mammals (Tachibana et al., 2001).

AN INTEGRATED SYSTEM FOR REGULATING APPETITE AND ENERGY BALANCE IN POULTRY

Figure 5 depicts a proposed regulatory system that integrates signals from peripheral tissues with specific brain centers to bring about short- and long-term changes in feed intake and energy expenditure to maintain body weight. Signals coming from the periphery include peptide hormones secreted by the gastrointestinal tract, adipose tissue, liver and the pancreas, as well as vagal afferents. Within the brain, the brainstem contains regions (satiety center) that receive and process signals from vagal afferent nerves and relay signals back to the GI tract via vagal efferents that control peripheral tissue functions and produce a sense of satiety. Contained within specific regions (nuclei) of the hypothalamus are two major feeding circuts (**Figure 4**). One set, comprised of neurons expressing genes for neuropeptide Y (NPY) and the agouti-related peptide (AGRP), is the orexigenic/anabolic arm of the regulatory system that stimulates appetite and reduces energy expenditure, thus promoting an increase in body weight. A second major set of neurons expressing proopiomelanocortin (POMC) that inhibits appetite and increases energy expenditure to bring about a reduction in body weight. Working together, the net effect of the combined activity of these two

412 feeding circuits is to establish a ‘set’ point for maintenance of body weight that can be
413 modulated to take into account changes in diet and environment.

414 The coordinate regulation of feed intake and energy balance involves the
415 integration of environmental cues (feed availability, feed composition, photoperiod,
416 temperature, stress) with internal physiological signals (hormone, metabolite levels,
417 energy stores). The brain plays a pivotal role in the process of integrating all of this
418 information and generating appropriate responses. A distributed neural network for the
419 control of feed intake and energy balance has been proposed that involves a central
420 processor (hypothalamus) and multiple negative feedback parallel processing loops
421 (Woods et al., 1998; McMinn et al., 2000; Blevins, et al., 2002; Berthoud, 2002).
422 Physiochemical interactions between specific neuropeptides and their receptors are
423 important to understanding the regulation of feeding behavior and energy balance. The
424 genes encoding neuropeptides and their respective receptors, expressed in
425 hypothalamic neurons as well as in peripheral tissues, are fundamental to creating a
426 sensing and signaling network that forms the basis for the regulation of feed intake and
427 energy balance. It has been suggested that α-MSH acting through the MC4-R serves
428 as an important central mediator for leptin action on feed intake and energy balance
429 (Forbes et al., 2001). Leptin signaling through the leptin receptor, enlists the response
430 of POMC-expressing hypothalamic neurons that gives rise to α-MSH and working
431 through the melanocortin receptor system. In addition to the primary circuit, leptin and
432 α-MSH can trigger additional neural pathways (neurons expressing other neuropeptides
433 such as thyrotropin-releasing hormone, and corticotrophin-releasing hormone) that also
434 affect feed intake and metabolic activity in addition to other roles involving the

435 hypothalamus-pituitary-thyroid or hypothalamus-pituitary-adrenal axes. Leptin is known
436 to repress the expression of NPY and AgRP genes in hypothalamic neurons in
437 mammals (Woods et al., 1998; McMinn et al., 2000; Blevins, et al., 2002). These
438 effects would repress the anabolic actions of NPY and AgRP neural pathways, and the
439 secondary pathways to which they connect. Similarly, working through melanocortin
440 receptors, α-MSH, produced by stimulated POMC-expressing neurons, would activate
441 additional neural pathways mediated by peptidergic neurons expressing corticotrophin-
442 releasing hormone and thyrotropin-releasing hormone genes that would work together
443 to decrease feed intake and increase metabolic activity, in part, via the hypothalamus-
444 pituitary-thyroid or hypothalamus-pituitary-adrenal axes (Woods et al., 1998; McMinn et
445 al., 2000; Blevins, et al., 2002). Thus, as plasma leptin levels rise in response to
446 elevated energy stores, catabolic pathways are activated and anabolic pathways are
447 repressed (**Figure 4**). The opposite occurs in the face of depleted energy stores and
448 lowered circulating leptin levels. Moreover, both leptin and α-MSH may also act directly
449 on peripheral tissues in light of the fact that their specific receptors are widely expressed
450 in these sites in chickens. The fact that additional downstream neural (second order
451 neurons) and neuroendocrine pathways are activated following the activation of the
452 NPY/AgRP and POMC (first order neurons) pathways helps to explain, in part, the
453 observed linkage of reproduction, immune system function, thermoregulation, bone
454 metabolism, and other physiological functions with energy status.

455 It is not known if circulating insulin levels reflect adipose tissue size in birds as
456 seems to be the case in mammals (Woods et al., 1998; McMinn et al., 2000; Blevins, et
457 al., 2002). Although insulin receptors have been identified in the central nervous

system of chickens (Simon and Leroith, 1986), there are no reports of the effects of central administration of insulin on feed intake in birds (Kuenzel, 1994). There is evidence for elevated circulating insulin levels in fed or feed-deprived chickens with lesions of the ventromedial hypothalamus, suggesting the production of metabolic obesity (Sonoda, 1983). Transient changes in plasma glucose level do not appear to alter feed intake in chickens (Simon et al., 2000). Therefore, a role for insulin as a hormonal signal of energy stores in poultry remains to be established.

FUTURE RESEARCH

Clearly one of the highest research priorities is to continue to identify, sequence, and functionally characterize unique genes and their products involved in the regulation of feed intake and energy balance in poultry. A comparative genomics approach will continue to be quite useful in identifying and characterizing avian homologues of previously identified mammalian genes. The key will be to determine any true differences that might exist in gene structure, expression, and function in birds as compared to mammals. In addition, genomic methods are required to identify and characterize novel genes that might play important regulatory roles heretofore undiscovered. The publication of a draft sequence for the chicken genome is an invaluable new resource that will aid in this endeavor. It is also important to carefully characterize specific gene sequences obtained from different populations of birds in order to begin to identify the presence of genetic mutations such as single nucleotide polymorphisms (SNPs) that may impact gene function. Our findings concerning the sequence variations in avian ghrelin genes is a good example of this type of approach (**Figure 1**). However, to date this has only been done to a very limited extent for other

genes involved in regulating feed intake and energy balance in poultry. DNA sequencing efforts will continue to generate new and more detailed avian sequence that will provide increasing amounts of valuable information concerning gene structure and function in poultry. Clearly, many challenges already exist and certainly leptin is a prime example of a gene that urgently needs further study to determine its true characteristics and functions specifically in poultry, if a viable leptin gene indeed exists.

Proteomic methodologies will be especially valuable in characterizing protein and peptide structures and identifying specific post-translational modifications in peptides and receptors that may be crucial to their normal functioning. Much of the evaluation of signaling proteins and peptides related to feed intake and energy balance to date has involved the effects of delivering various synthetic or recombinant homologues or analogs peripherally (ip, iv) or centrally (icv) to birds to determine their bioactivity. Unfortunately, conflicting results with respect to efficacy have sometimes been obtained and it is difficult to ascertain if this is due to legitimate differences in activity or is related to discrepancies in the structures of the molecules being administered. Advances in proteomic technologies will facilitate discovery of the native structures of signaling peptides and their receptors containing unique modifications. Examples of such important modifications include: the fatty acid modification of ghrelin (Kaiya et al., 2002, Saito et al., 2002), phosphorylation of specific amino acid residues of the leptin receptor (Ohkubo et al., 2000; Horev et al., 2000; Richards and Poch, 2003), specific truncation or modification of peptides at the amino- or carboxyl-ends, and proteolytic processing of prohormone peptide precursors.

Specific genetic lines of mice such as those with spontaneous mutations in leptin (*ob/ob*), or leptin receptor (*db/db*) genes have proven to be highly valuable research tools for elucidating the regulatory mechanisms involved in controlling appetite and energy balance. Unfortunately, there are currently no such avian models. However, specific populations of birds (e.g., obese vs. lean, fast vs. slow growing, layers vs. broilers, etc.) developed through the use of classical genetic selection techniques continue to provide a useful testing ground for discovery and evaluation of specific genes that function in the regulation of feed intake and energy balance. In addition, specific stages of the lifecycle such as post-hatch and during the transition to egg production are key times to be investigated with respect to appetite control and energy homeostasis.

Transgenic mice have also proven to be invaluable in evaluating the functions and effects of individual genes in mammals. The use of knockout mouse models has been particularly useful in further defining the nature of the mechanisms regulating feed intake and energy homeostasis. A good example of this is the production of NPY knockout mice that display normal feed intake and energy balance phenotypes (Palmiter et al., 1998). This particular model highlights the redundancy in networks controlling feed intake and energy balance and alternative pathways identified in such models represent potentially useful areas for future investigations in avian species. There has been some discussion of using cellular and molecular biology methods to introduce specific genes into poultry with cloning being used to propagate desirable genotypes (Etches, 2001). The development and testing of genetically modified avian

525 models specifically related to different aspects of the control of feed intake and energy
526 balance would undoubtedly yield new information.

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Table 1
Adipokines: Factors produced and secreted by adipose tissue

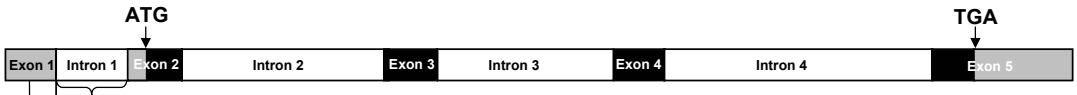
Factor	Function
1. Autocrine/paracrine factors also produced by non-adipose tissue:	
Angiotensinogen	Cardiovascular function
Insulin-like growth factor I (IGF-I)	Growth factor
Vascular Endothelial Growth factor (VEGF)	Growth factor
Nerve Growth factor (NGF)	Growth factor
Lipoprotein Lipase (LPL)	Lipolysis/triglyceride metabolism
Plasminogen Activator Inhibitor-1 (PAI-1)	Cardiovascular function
Interleukin-1 (IL-1)	Cytokine
Interleukin-6 (IL-6)	Cytokine
Tumor Necrosis factor α (TNF-α)	Cytokine
Sex steroids	Reproduction
2. Adipose-specific endocrine factors:	
Leptin	Appetite/energy homeostasis/body weight
Resistin	Insulin resistance
Adiponectin (ACRP30)	Energy homeostasis/body weight
Adipsin	Fatty acid metabolism
Adiponutrin	Energy homeostasis
Acylation-Stimulating Protein (ASP)	Fatty acid esterification
Fasting-Induced Adipose Factor	Metabolic regulation

Table 2
Peptide signals that affect appetite and energy balance[1]

Orexigenic (Anabolic)	No Effect	Anorexigenic (Catabolic)
	Birds	
Neuropeptide Y (NPY)	Melanin Concentrating Hormone (MCH)	α-Melanocyte Stimulating Hormone (α-MSH)
Agouti-related Peptide (AGRP)	Orexins (A&B)	Leptin (?)
Peptide YY (PYY)	Galanin	Ghrelin
Pancreatic Polypeptide (PP)	Motilin	Glucagon-like Peptide 1 (GLP-1)
	Leptin (?)	Cholecystokinin (CCK)
		Bombesin/Gastrin Releasing Peptide (GRP)
		Growth Hormone Releasing Factor (GHRH)
	Mammals	
Neuropeptide Y (NPY)		α-Melanocyte Stimulating Hormone (α-MSH)
Agouti-related Peptide (AGRP)		Leptin
Ghrelin		Glucagon-like Peptide 1 (GLP-1)
Melanin Concentrating Hormone (MCH)		Cholecystokinin (CCK)
Orexins (A&B)		Bombesin/Gastrin Releasing Peptide (GRP)
Galanin		Neurotensin
Motilin		Somatostatin (SRIH)
Growth Hormone Releasing Factor (GHRH)		Thyrotropin-Releasing Hormone (TRH)
		Corticotropin-Releasing Hormone (CRH)
		Peptide YY (PYY)
		Pancreatic Polypeptide (PP)

[1] Based on information taken from: Woods et al., 1998; Richards, 2003.

AVIAN GHRELIN GENE STRUCTURE



Sequence Variations:

```
                    Exon 1                                                  Exon 2
Broiler:            agaaaacaca----------------tt--------------tgaagcactg
White Leghorn:      agaaaacaca----------------tt--------------tgaagcactg
Red Jungle Fowl:    agaaaacaca----------------tt--------------tgaagcactg
Turkey:             agaaaacacaAAACAACTGCATATCAttCAAATCAGGAGAAAtaaagcaccA
Goose:              agaaatcactG----------------tt--------------tgaAcaccG
Emu:                agaaaacacG----------------tt--------------tgaAcaccG
```

```
Broiler:            tttctctctg--------tctggtccag
White Leghorn:      ttcctctctgCTAACCTGtctggtccag
Red Jungle Fowl:    tttctctctg--------tctgttccag
Turkey:             tttctctctgCTAACCTAtctgttccag
Goose:              ttcctctctgCTAACCCAtctgccgccag
Emu:                ...ctctctgCTAGCCCAtctgcttcag
```

Species Comparisons:

Gene Feature	Chicken[1]	Turkey[2]	Mouse[3]	Human[4]
1. Exon Size (bp)				
Exon 1	127	127	19	-
Exon 2[a]	136	166	133	140
Exon 3[a]	114	114	117	117
Exon 4[a]	109	109	109	109
Exon 5[a]	358	353	143	144
2. Intron Size (bp)				
Intron 1	183	80	409	-
Intron 2	524	528	93	194
Intron 3	463	450	1942	2948
Intron 4	692	688	783	809
3. mRNA Size (bp)				
mRNA (cDNA)	844[b]	869	521	510
Coding Region	351	351	354	354
5'-UTR	155[b]	185	44	32
3'-UTR	338	333	123	124

Sequence data was obtained from GenBank Deposits as follows: [1]Chicken (AY299454 & AY303688), [2]Turkey (AY333783 & AY497549), [3]Mouse (NM_021488 & AB060078), [4]Human (AB029434 & AF296558).

[a] These exons contain coding sequence.

[b] Values are given for Leghorn. Broiler chickens lack an 8 bp sequence feature contained in exon 1 (5'-UTR).

Figure 1. Proposed structure of the avian ghrelin gene based on sequence data obtained for the chicken (GenBank Accession No. AY303688) and turkey (GenBank Accession Nos. AY497549, AY333783). The positioning and size (in bp) of each of the 5 exons and 4 introns is indicated. The positions of the start codon (ATG, located in exon 2) and stop codon (TGA, located in exon 5) are also indicated with the black boxes designating coding region. The location of different insertion/deletion (INDEL) features in the first exon which contains 5'-untranslated region and at the junction of exons 1 and 2 for different avian species is depicted. A species comparison of gene features including exon and intron sizes and mRNA features is also included in the table.

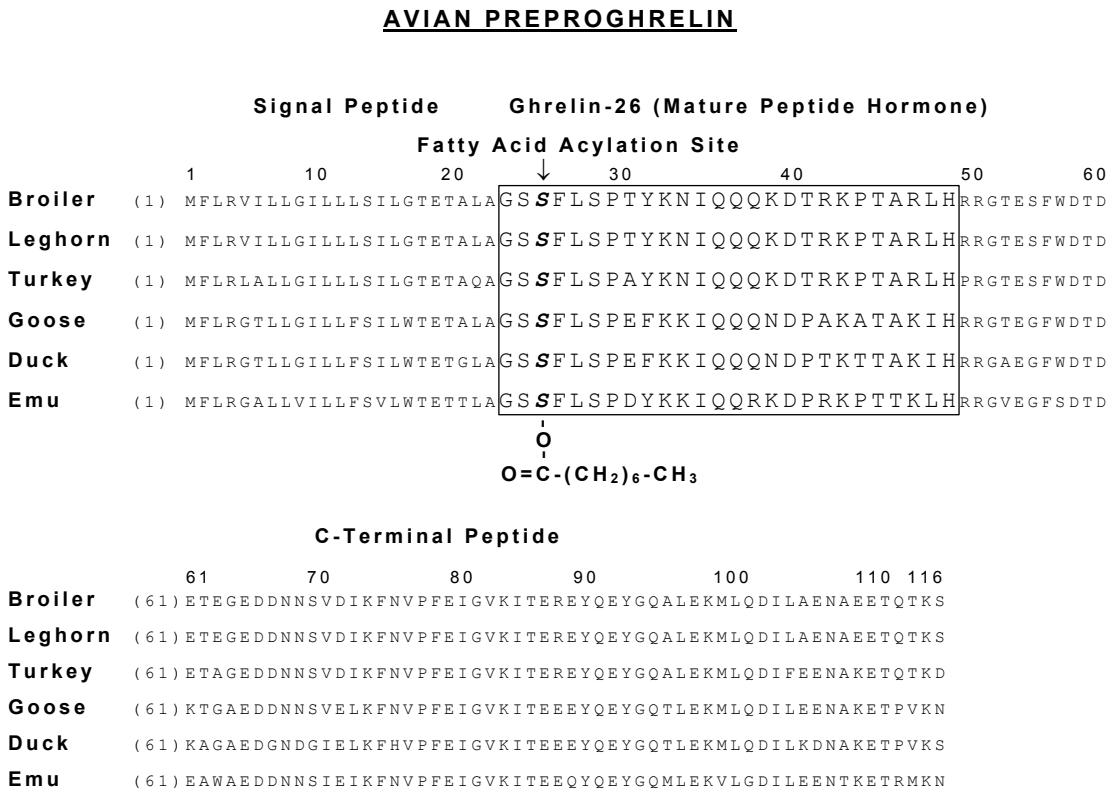


Figure 2. Amino acid comparisons of preproghrelin proteins (116 AA) for different avian species. The locations of the signal peptide (23 AA), mature ghrelin peptide (26 AA) and the C-terminal peptide (67 AA) are indicated. Also indicated is the site within the mature ghrelin peptide (serine 3) for acylation by octanoic acid or decanoic acid required for growth hormone secretagogue receptor (GHS-R) binding and signaling.

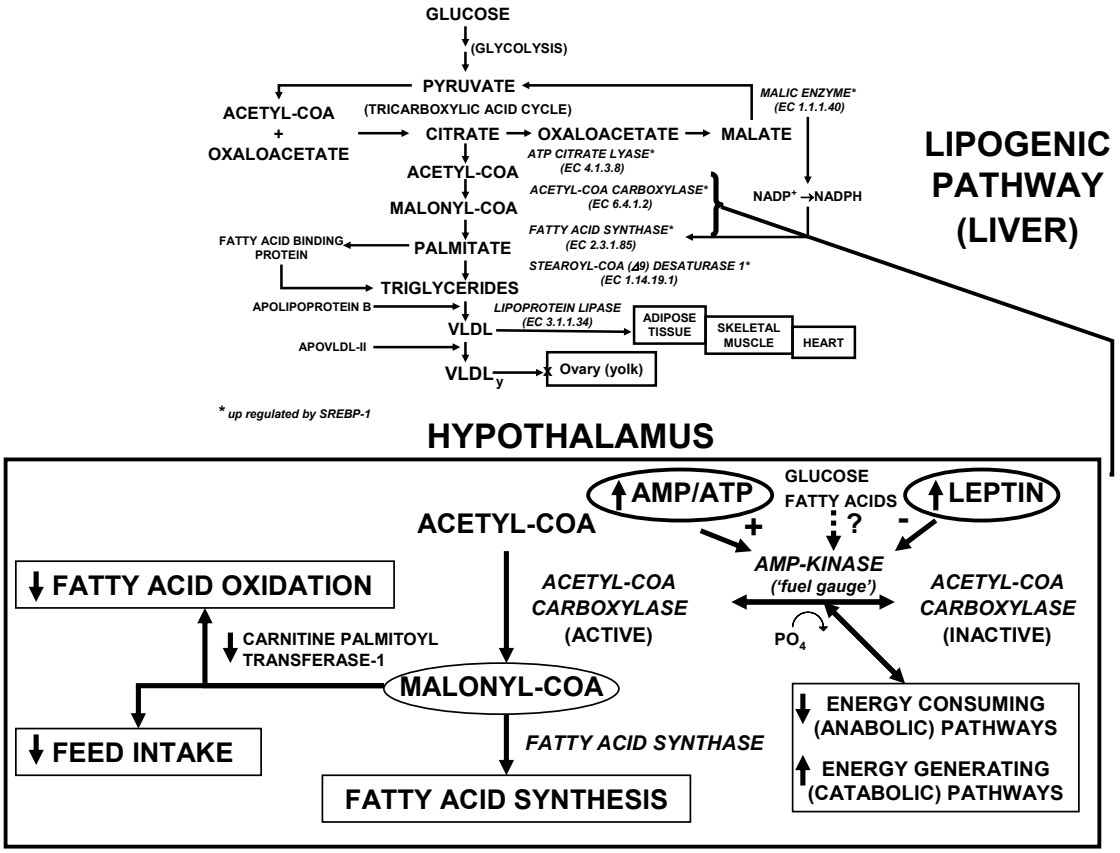


Figure 3. The lipogenic metabolic pathway (upper portion) responsible for the *de novo* production of triglyceride from glucose in liver. Specific enzymes (italics) are shown next to the steps in the reaction that they catalyze. Also shown, is the role of the enzyme AMP-activated protein kinase (AMP-kinase) as a cellular 'fuel gauge' by sensing the cellular energy level as indicated by the AMP/ATP ratio in the hypothalamus. When activated, AMP-kinase activates hypothalamic pathways (catabolic) that generate energy while inhibiting energy consuming (anabolic) pathways. AMP-kinase also influences the level of hypothalamic malonyl-COA by modulating the level of activity of acetyl-COA carboxylase, the rate-limiting enzyme of the lipogenic pathway. An accumulation of malonyl-COA then activates anorexigenic hypothalamic circuits that cause a decline in feed intake. An accumulation of malonyl-COA can also inhibit the activity of carnitine palmitoyl transferase-1, the rate limiting enzyme in the fatty acid oxidation pathway. An increase in leptin level has been reported to inhibit hypothalamic AMP-kinase activity.

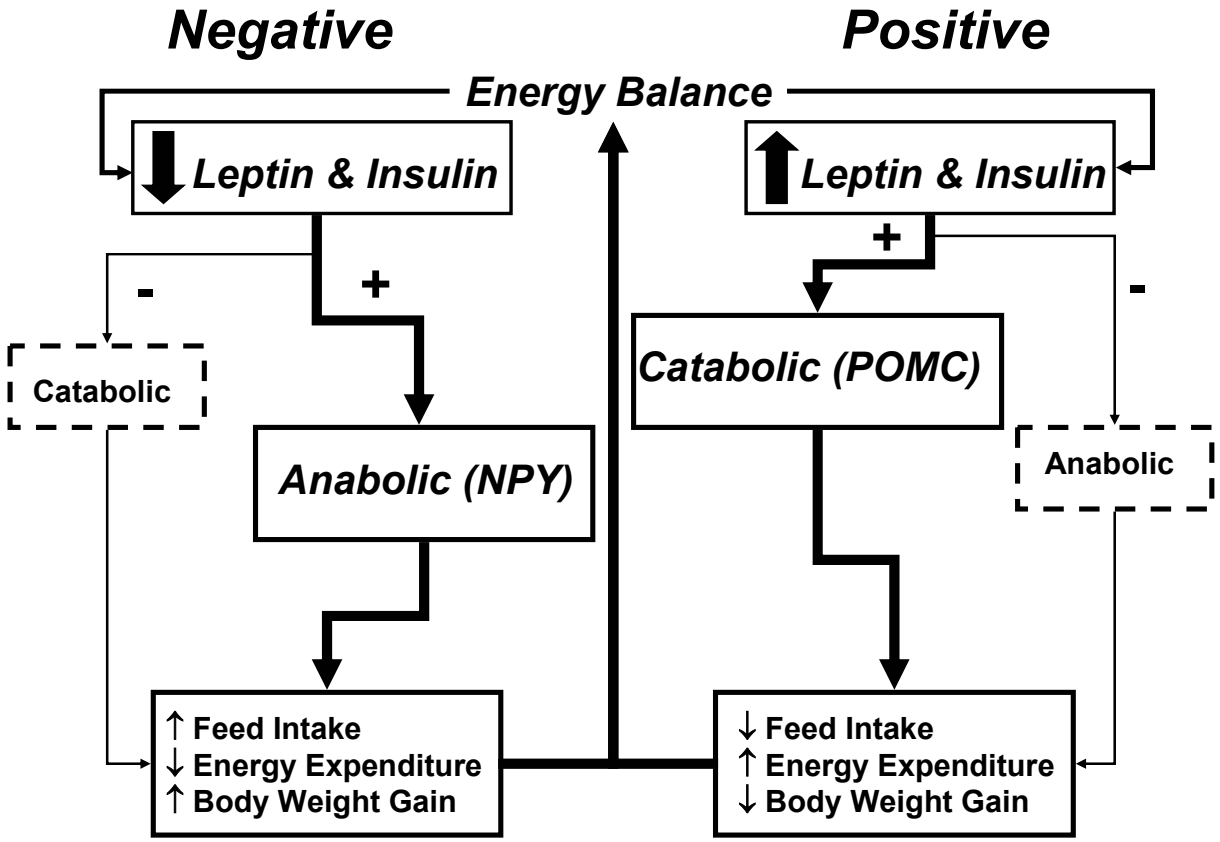


Figure 4. A proposed mechanism for the long-term regulation of energy balance and body weight. Two specific sets of hypothalamic neural circuits, designated as anabolic (neuropeptide Y expressing, NPY) or catabolic (proopiomelanocortin expressing, POMC) are activated in response to changes in energy status. The net effect is to produce the appropriate changes in feed intake and energy expenditure to bring the bird back into a state of energy balance. Circulating levels of the hormones leptin and insulin represent potential 'energy sensing' signals to the hypothalamus that determine the level of activity in both the anabolic and catabolic pathways. Acting together, these important negative feedback circuits help ensure stability in body weight over the long-term.

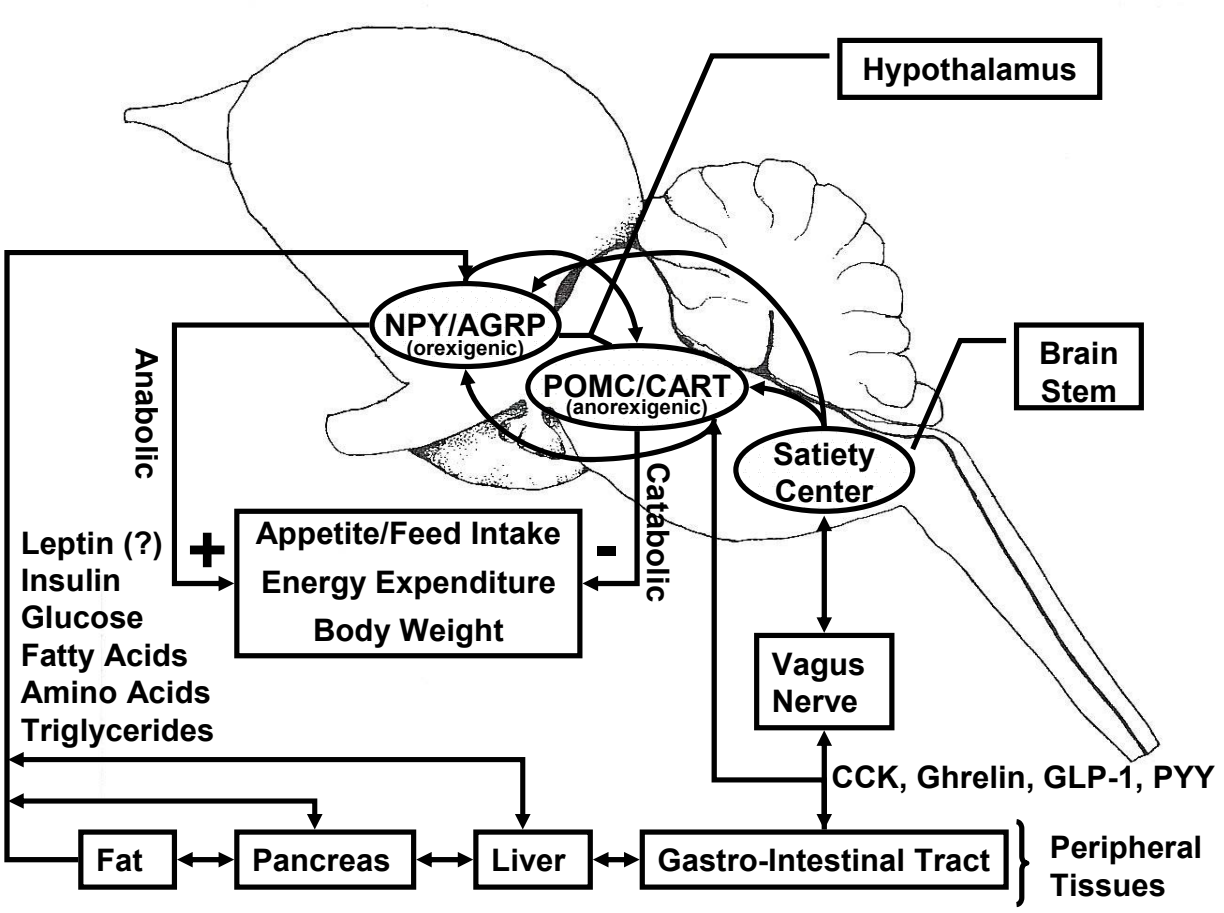


Figure 5. A proposed model describing the long-term regulation of appetite and energy balance to achieve a stable body weight in poultry that integrates peripheral tissue and central nervous system circuits regulated by hormonal, neural, neuroendocrine and nutrient signaling mechanisms.